Published in final edited form as:

Mamm Genome. 2011 February ; 22(1-2): 43–54. doi:10.1007/s00335-010-9293-3.

The immunity-related GTPases in mammals: a fast-evolving cell-autonomous resistance system against intracellular pathogens

Julia P. Hunn,

Institute for Genetics, University of Cologne, Zulpicher Str. 47a, 50674 Cologne, Germany

Carl G. Feng,

Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Alan Sher, and

Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Jonathan C. Howard

Institute for Genetics, University of Cologne, Zulpicher Str. 47a, 50674 Cologne, Germany

Jonathan C. Howard: j.howard@uni-koeln.de

Abstract

The immunity-related GTPases (IRGs) belong to the family of large, interferon-inducible GTPases and constitute a cell-autonomous resistance system essential for the control of vacuolar pathogens like *Toxoplasma gondii* in mice. Recent results demonstrated that numerous IRG members accumulate collaboratively at the parasitophorous vacuole of invading *T. gondii* leading to the destruction of the vacuole and the parasite and subsequent necrotic host cell death. Complex regulatory interactions between different IRG proteins are necessary for these processes. Disturbance of this finely balanced system, e.g., by single genetic deficiency for the important negative regulator *Irgm1* or the autophagic regulator *Atg5*, leads to spontaneous activation of the effector IRG proteins when induced by IFN γ . This activation has cytotoxic consequences resulting in a severe lymphopenia, macrophage defects, and failure of the adaptive immune system in *Irgm1*-deficient mice. However, alternative functions in phagosome maturation and induction of autophagy have been proposed for *Irgm1*. The IRG system has been studied primarily in mice, but IRG genes are present throughout the mammalian lineage. Interestingly, the number, type, and diversity of genes present differ greatly even between closely related species, probably reflecting intimate host-pathogen coevolution driven by an armed race between the IRG resistance proteins and pathogen virulence factors. IRG proteins are targets for polymorphic *T. gondii* virulence factors, and genetic variation in the IRG system between different mouse strains correlates with resistance and susceptibility to virulent *T. gondii* strains.

Introduction

The immunity-related GTPases (IRG proteins, formerly called p47 GTPases) were first described in the 1990s as genes strongly induced by infection via interferon gamma (IFN γ) (and to a lesser extent by type I IFN) in mice (Boehm et al. 1998; Carlow et al. 1995; Gilly and Wall 1992; Lafuse et al. 1995; Sorace et al. 1995; Taylor et al. 1996). Targeted deletions

pioneered by Gregory Taylor established an essential function of the IRG proteins in early pathogen resistance (Collazo et al. 2001; Liesenfeld et al. unpublished data; Taylor et al. 2000). These and subsequent studies implicated the IRGs in resistance against a wide variety of intracellular pathogens, including *Toxoplasma gondii*, *Leishmania major*, *Trypanosoma cruzi*, *Chlamydia trachomatis*, *C. psittaci*, *Mycobacterium tuberculosis*, *M. avium*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Legionella pneumophila* (Coers et al. 2008; Collazo et al. 2001; Feng et al. 2004; Henry et al. 2007, 2009; Liesenfeld et al. unpublished data; MacMicking et al. 2003; Miyairi et al. 2007; Santiago et al. 2005; Taylor 2007; Taylor et al. 2000). The proposed mechanisms of IRG-mediated resistance range from enhancement of phagosome maturation and induction of autophagy in mycobacterial immunity (Gutierrez et al. 2004; MacMicking et al. 2003) to destruction of pathogen-containing vacuoles and induction of host cell necrosis in immunity against *T. gondii* (Gutierrez et al. 2004; MacMicking et al. 2003; Martens et al. 2005; Zhao et al. 2009b).

IRG proteins are large GTPases containing a Ras-like G domain and a helical domain combining N- and C-terminal elements (Ghosh et al. 2004) (Fig. 1). Most of the IRG proteins possess the canonical and highly conserved GxxxxGKS/T P-loop sequence in the first nucleotide-binding motif (G1) and form the “GKS” subfamily (Bekpen et al. 2005; Boehm et al. 1998). The GMS subfamily IRG proteins, however, contain the altered GxxxxGMS motif and other sequence features that distinguish them from the rest of the IRG family (Bekpen et al. 2005; Boehm et al. 1998). In other GTPase families, mutation of the conserved G1 motif lysine results in impaired nucleotide binding (Pitossi et al. 1993; Praefcke et al. 2004; Sigal et al. 1986), but there is some evidence that the GMS proteins are able to bind guanine nucleotides despite their unusual G1 motif (Taylor et al. 1997). The structure and biochemical properties of the only IRG protein that has been characterized in detail so far, the GKS subfamily member Irga6 (originally called IIGP1), are distinct from the small, Ras-like GTPases and reminiscent of the large dynamin-like GTPases (Uthaiiah et al. 2003). The latter group includes the other two large, interferon-inducible GTPase families implicated in host resistance against intracellular pathogens: the guanylate-binding proteins (GBPs or p65 GTPases) and the Mx proteins (Degrandi et al. 2007; Praefcke and McMahon 2004).

Dynamin-like GTPases and Irga6 have micromolar nucleotide-binding affinities and undergo pronounced conformational changes upon GTP-dependent activation (Praefcke and McMahon 2004; Uthaiiah et al. 2003). No external GTPase-activating proteins (GAPs), which are essential for the activation of Ras-like GTPases (Boguski and McCormick 1993), have been identified for any of the large GTPases. Instead these proteins self-activate by GTP-dependent homo-oligomerization (Binns et al. 1999; Gao et al. 2010; Prakash et al. 2000; Tuma and Collins 1994; Uthaiiah et al. 2003; Warnock et al. 1996). In the case of Irga6, GTP-dependent homo-oligomers form in vitro via a G domain-G domain interaction involving the bound nucleotide and a second uncharacterized interface (Pawlowski et al. unpublished data). In the absence of any evidence for GAP or GEF (guanine nucleotide exchange factor) proteins, the tendency to activate by oligomerization suggested that the IRGs would need a negative regulator that functions as a GDI (guanine nucleotide dissociation inhibitor) in vivo following IFN γ induction but prior to infection. The detailed studies of the cell biology of the IRGs described in the next section revealed that this is indeed the case.

Cell biology and function

Since the early work on IRG proteins was focused on infection studies of mice deficient for individual *IRG* genes, the elucidation of the molecular mechanisms by which the IRG proteins mediate resistance was lagging behind and is still far from complete. The first

crucial step was the discovery of the cell autonomous nature of the IRG resistance (Halonen et al. 2001; Henry et al. 2009; Konen-Waisman and Howard 2007; Martens et al. 2005). Based on subsequent studies, three distinct models of IRG function have been proposed: (1) the destruction of pathogen-containing vacuoles by a joint action of at least seven, if not all, of the IRG proteins culminating in the destruction of the pathogen and necrotic host cell death (Hunn et al. 2008; Khaminets et al. 2010; Ling et al. 2006; Martens et al. 2005; Zhao et al. 2009b); (2) the enhancement of phagosome maturation by the IRG protein Irgm1 (LRG-47) (MacMicking et al. 2003); and (3) the induction of autophagy by IRG proteins (Gutierrez et al. 2004; Singh et al. 2006). Though not necessarily mutually exclusive, these three models of IRG function raise some interesting controversies.

It is important at this stage to introduce the intracellular protozoan parasite *T. gondii* in more detail, since it features strongly in the rest of this review. A central point for the research we describe is that *T. gondii* is a natural and abundant parasite of wild mice. Thus, virulence and resistance phenomena analyzed in mice in vivo and in cellular systems in vitro reflect the adaptive behavior of a coevolving host-parasite system in a reasonably natural setting. The definitive hosts of *T. gondii*, in which the sexual cycle is completed, are members of the *Felidae*, the true cats. In much of the modern world, this effectively reduces to *Felis catus*, the domestic cat. Haploid products of meiosis are distributed in cat feces in environmentally stable oocysts in enormous numbers and are inadvertently ingested during foraging by mammals and birds belonging to a diverse range of species. In these warm-blooded hosts the oocysts rupture and release rapidly replicating tachyzoites that invade the intestinal mucosa. Tachyzoites can penetrate nucleated cells of all types by an active entry mechanism distinct from phagocytosis that results in the formation of a nonfusogenic intracellular vacuole, the parasitophorous vacuole (PV), surrounded by a lipid membrane (PVM) largely derived from the plasma membrane but modified by the addition of several proteins derived from secretory organelles of the parasite. After an initial phase of rapid intracellular replication and expansion as tachyzoites, the parasites migrate to muscle and the central nervous system where a life-cycle change to slowly replicating bradyzoites occurs. The bradyzoite-infected cells then form cysts that persist in a latent state, often for the life of the host. The life cycle is completed when the infected host is eaten by a cat. The cysts break down in the cat intestine and the enclosed bradyzoites penetrate feline intestinal mucosal cells and initiate gametogenesis. Most research on *T. gondii* has been conducted on the rapidly replicating tachyzoite phase because it is easy to maintain in in vitro mammalian cell culture. The processes of entry, PV formation, replication, and ultimate egress can all be studied in detail. This has provided the analytical systems in which IRG protein function can be studied.

The first model of IRG function is based on the extensive work that has been performed by several groups on IRG-mediated resistance against *T. gondii*. At least seven IRG proteins localize to the *T. gondii*-containing PV shortly after infection (Hunn et al. 2008; Khaminets et al. 2010; Lilue et al. unpublished data; Ling et al. 2006; Martens et al. 2005; Melzer et al. 2008; Zhao et al. 2009a). The coating of PVs with IRG proteins is rapid (complete in less than 1 h), collaborative, and hierarchical, with Irgb6 (TGTP) and Irgb10 functioning as pioneers in the sense of loading first (Hunn et al. 2008; Khaminets et al. 2010), and requires complex regulatory interactions between IRG family members (Hunn et al. 2008; Papic et al. 2008). In the IFN-induced, uninfected cell, GKS IRG proteins are kept in the inactive state via GDP-dependent interaction with the negative regulatory GMS IRG proteins Irgm1, Irgm2 (GTPI), and Irgm3 (IGTP) (Hunn et al. 2008). The GMS proteins hereby function as guanine nucleotide dissociation inhibitors (GDIs) for the GKS proteins. Upon infection with *T. gondii* and other vacuolar pathogens [like *Chlamydia trachomatis* (Coers et al. 2008)], GKS proteins lose their interaction with the GMS proteins and accumulate at the PVM in the active GTP-bound state (Hunn et al. 2008; Papic et al. 2008). This leads to vesiculation and rupture of the PV, death of the parasite (Ling et al. 2006; Martens et al. 2005; Melzer et al.

2008), and subsequent necrotic host cell death (Zhao et al. 2009b) (Fig. 2). The molecular mechanisms of these processes are still poorly understood, but an extraction of lipid from the PVM via dynamin-like vesiculation (Bashkirov et al. 2008; Pucadyil and Schmid 2008; Sweitzer and Hinshaw 1998) leading to increased tension in the membrane and ultimate rupture is plausible. This notion is consistent with the observation that the vacuoles and enclosed *T. gondii* tend to round up before rupture, that rupture occurs only at one place, and that immediately after rupture the previously enclosed *T. gondii* resumes its banana shape (Zhao et al. 2009b). When the regulatory balance of IRG proteins is disturbed, e.g., by absence of the negative regulator Irgm1 (discussed in detail in Hunn and Howard 2010), the IRG effector proteins activate prematurely and form aggregates that are toxic for cells. Under these circumstances the vacuolar localization of IRG proteins is impaired and the IRG resistance system fails (Henry et al. 2009; Khaminets et al. 2010; Zhao et al. 2008) (Könen-Waisman and Taylor, unpublished data).

T. gondii strains exhibit pronounced virulence differences in mice. A single parasite of a virulent type I strain can kill a mouse while several orders of magnitude higher doses of avirulent type II or III strains are tolerated (and lead to chronic infection) (Araujo et al. 1976; Boothroyd and Grigg 2002; Howe and Sibley 1995). These virulence differences are reflected in the IFN γ -induced cell-autonomous resistance in vitro (Khaminets et al. 2010; Zhao et al. 2009c). The replication of avirulent strains is strongly inhibited by IFN γ , while type I strains are markedly less affected. This deficiency in IFN γ -mediated control is associated with a striking failure of IRG proteins to accumulate on the PVM (Khaminets et al. 2010; Zhao et al. 2009a, c). This is at least in part due to the polymorphic kinase ROP18 (Taylor et al. 2006), secreted from the rhoptry secretory organelles of virulent *T. gondii* strains, which phosphorylates the GKS IRG proteins Irga6, Irgb6, and Irgb10 (Steinfeldt et al. unpublished data; Fentress et al. unpublished data). For Irga6, it has been shown that the phosphorylation is at two threonine residues in the P loop of the nucleotide binding site, thereby inactivating the protein and reducing vacuolar loading. Thus, virulence correlates with the ability to inactivate the IRG effector proteins efficiently.

A similar correlation of pathogen virulence with the ability to counteract the IRG resistance system in mice was observed for the Gram-negative bacterium *Chlamydia*. Vacuoles (termed inclusions) containing the human pathogenic strain *C. trachomatis* accumulate multiple IRG proteins and are destroyed, while the mouse pathogenic strain *C. muridarum* inclusions are not targeted by the IRG proteins and are not controlled by IFN γ (Al-Zeer et al. 2009; Coers et al. 2008). The virulence determinant of *C. muridarum* that counteracts the IRG system and its mechanism of action remains to be identified.

The second model of IRG function relies on the observed phagosomal localization of Irgm1 (Martens et al. 2004). Phagosome maturation is delayed in *Irgm1*-deficient macrophages and correlates with the loss of control of *Mycobacterium tuberculosis* in vivo and in vitro, while Irgm3 and Irgd deficiency cause no such defect (Feng et al. 2008a; MacMicking et al. 2003). However, two recent studies demonstrated that *Irgm1*-deficient macrophages display profound generalized cellular defects (Henry et al. 2009, 2010) that may be due to the formation of toxic aggregates of GKS effector IRG proteins in the absence of the important negative regulator, Irgm1. In line with this, the cellular defects as well as the loss of resistance against *Mycobacteria* revert to normal in *Irgm1/Irgm3* double-deficient cells accompanied by much reduced expression levels of the GKS proteins (Feng, Taylor and Sher unpublished data; Henry et al. 2009). Thus, it seems unlikely that the delay in phagosome maturation observed in *Irgm1*-deficient cells is directly linked to a specific function of Irgm1 in this process (for detailed discussion see Hunn and Howard 2010).

The third model, the induction of autophagy by the IRG protein Irgm1 causing pathogen death and destruction (Gutierrez et al. 2004; Singh et al. 2006), needs to be reevaluated for the same reasons. In macrophages, expression of Irgm1 was reported to induce, and RNAi-mediated knockdown to reduce, autophagosome formation (Gutierrez et al. 2004; Singh et al. 2006). In contrast, absence of Irgm1 resulted in autophagosome formation in activated, IFN γ -induced CD4⁺ T cells (Feng et al. 2008b). These cell-type-specific differences in the effects of Irgm1 on autophagy remain to be explained. Interestingly, there is further evidence for marked cell-type-specific differences: while induction of autophagic killing of pathogens has been linked to Irgm1 repeatedly in macrophages (Gutierrez et al. 2004; Singh et al. 2006), this could not be observed in astrocytes or fibroblasts (Halonen 2009; Ling et al. 2006; Martens et al. 2005; Zhao et al. 2008). However, autophagy may contribute somewhat to the clearance of dead pathogens in these cells. Given the absence of Irgm1-induced macroautophagy from all analyzed cell types other than macrophages, it is conceivable that autophagy induction in the context of an IRG-driven resistance is a mechanism confined to specialized phagocytes, perhaps favoring efficient antigen presentation (Bougneres et al. 2009; Halonen 2009). It is likely that this functions downstream of or in parallel with the IRG-induced pathogen death described by the first model.

Strikingly, the IFN γ -mediated control of *T. gondii* and *C. trachomatis* replication is lost in cells deficient for the autophagic regulator Atg5 (Al-Zeer et al. 2009; Konen-Waisman and Howard 2007) and the IRG proteins fail to target the PVM of infecting a virulent parasites (Khaminets et al. 2010; Zhao et al. 2008). It has been suggested that Atg5 is necessary for the trafficking of IRG proteins to the vacuole (Zhao et al. 2008). However, the absence of Atg5 leads to premature activation of the IRG effector proteins in the cytoplasm by an unknown mechanism (Khaminets et al. 2010) similar to what was observed in *Irgm1*-deficient cells (Henry et al. 2009). This activation by itself is sufficient to explain the striking failure of the proteins to translocate to the PVM.

Furthermore, it remains to be clarified whether autophagy in the context of a certain infection is indeed a cell-autonomous resistance mechanism or whether it serves as a disposal system to clear pathogens killed by another resistance mechanism (as discussed in Halonen 2009; Ling et al. 2006; Martens et al. 2005). Recently, it even has been suggested that induction of autophagy by *T. gondii* in infected host cells promotes parasite growth by supplying nutrients to the parasite (Orlofsky 2009; Wang et al. 2009). Thus, the role of autophagy in this infection is far from clear and deserves further investigation.

Mechanisms of IRG protein action in vivo are considered in more detail in the next section.

In vivo role of IRG proteins in host defense and autoimmunity

As noted above, the function of the IRG proteins in host resistance in vivo was not formally established until mice in which *Irgm1*, *Irgm3*, *Irgd*, and *Irga6* had been genetically deleted became available. Taylor et al. (2000) first showed that *Irgm3* knockout mice are significantly more susceptible to *T. gondii* infection than their wild-type counterparts. Since then, *IRG*-deficient animals have been tested with a variety of experimental protozoan, bacterial, and viral infections that require IFN γ signaling for host resistance (Taylor et al. 2004, 2007) (Table 1). While displaying normal resistance to certain viral infections, IRG knockout mice were shown to display dramatic gene- and pathogen-specific differences in susceptibility to intracellular bacterial and parasitic pathogens. For example, *Irgm3*-deficient mice showed increased susceptibility to *T. gondii* (Taylor et al. 2000) but normal resistance to *T. cruzi*, *Salmonella*, and mycobacterial infection. In contrast, *Irgm1*-deficient mice are highly susceptible to all intracellular bacteria and protozoa that have been examined to date, demonstrating a functional distinction between these two IRG proteins in host defense.

Because Irgm1 and Irgm3 enhance intracellular parasitic and bacterial killing by promoting phagolysosomal maturation and autophagy in vitro (Gutierrez et al. 2004; Ling et al. 2006; MacMicking et al. 2003; Singh et al. 2006), these GTPases were originally proposed to be microbicidal effector molecules that are complementary to the activity of nitric oxide in IFN γ -activated macrophages, although, as discussed above, this is an oversimplified view of the actual mechanisms involved. Moreover, from an in vivo perspective, it is now clear that the increased susceptibility to infection seen in *Irgm1*-deficient mice may stem in part from effects on the host immune system distinct from the proposed role of the gene in pathogen killing. Thus, in the case of persistent *M. avium* (Feng et al. 2004) and *T. cruzi* (Santiago et al. 2005) infections, *Irgm1*^{-/-} mice were found to develop a profound pancytopenia associated with impaired expansion of hematopoietic stem and progenitor cell populations (Feng et al. 2008a). These global defects could result in compromised host resistance due to impaired generation or survival of host immune effector cells.

Focusing on mature CD4⁺ T cells in the Th1 response to infection, it was found that Irgm1 is critical for the survival of mature lymphocytes since in the absence of Irgm1, IFN γ induced death of these cells. Moreover, the reduction in lymphocytes and increased mortality previously observed in *M. avium*-infected *Irgm1*^{-/-} animals was completely abrogated in *Irgm1*^{-/-}*Ifng*^{-/-} mice (Feng et al. 2008b). Independently, Taylor's group also discovered that the defective bacterial control and granulomatous response displayed by *S. typhimurium*-infected *Irgm1*^{-/-} mice is reversed in *Irgm1*^{-/-}*Irgm3*^{-/-} animals (Henry et al. 2009). Recent studies have revealed that both the hematopoietic and host-resistance defects of *M. avium*-infected *Irgm1* knockout mice are rescued in *Irgm1*^{-/-}*Irgm3*^{-/-} mice (Feng et al. unpublished data). Together, these in vivo observations reveal a regulatory function for Irgm1 in the IFN γ -dependent host response to chronic bacterial infection and argue that in the absence of Irgm1, dysregulated cellular responses, rather than defective effector cell function, are sufficient to explain impairment in host resistance observed in vivo. Whether this involves the negative regulation by Irgm1 of other IRG members, as proposed from the cell biological studies described above (Hunn and Howard 2010; Hunn et al. 2008), remains to be investigated.

A role for IRG function in host resistance and disease in humans has been inferred from forward genetic studies. As discussed below, only one IRG homolog, IRGM, is present in the human genome and expressed outside the germline. Furthermore, IRGM is severely truncated compared with a typical IRG gene product (Bekpen et al. 2005). However, based on in vitro studies, this protein has been proposed to participate in antibacterial microbicidal function (Singh et al. 2006). In 2007, two single nucleotide polymorphism (SNP)-based, genome-wide association studies independently demonstrated strong association signals linking IRGM to Crohn's disease (CD) susceptibility (Consortium WTCC 2007; Parkes et al. 2007). Since then, numerous follow-up studies, including a recent genome-wide association study of copy number variants (Craddock et al. 2010), have confirmed the genetic association of IRGM variants with CD. In addition, two recent reports have described significant associations between IRGM SNP and tuberculosis (Che et al. 2010; Intemann et al. 2009). The mechanisms underlying this genetic association with IRGM and disease susceptibility have yet to be defined. In the case of CD, other genetic loci involved in autophagy [e.g., Atg16L1 (Cadwell et al. 2008; Consortium WTCC 2007)] have been linked to disease expression and therefore it is possible that IRGM may exert its function through this mechanism (McCarroll et al. 2008). Intestinal bacteria are thought to play a major role in the pathogenesis of CD, and mutations in IRGM that lead to defective autophagic elimination of intracellular bacteria could provide an explanation for the observed genetic findings, although as discussed below controversy still surrounds the issue of whether human IRGM, although homologous to the three mouse GMS proteins, is a functional protein.

Evolution of the IRGs

The first detailed genomic analysis of IRG genes was performed in inbred laboratory mice. Altogether there are 21 IRG genes in the C57/BL6 mouse (Bekpen et al. 2005). Seventeen genes possess the typical structure for IRG genes: one long coding exon containing the whole open reading frame (ORF) of roughly 1.2-kb length with one or two noncoding 5' exons. These classical IRG genes encode proteins of 410–420 amino acids and 47-kDa molecular weight. The remaining four genes are head-to-tail arrangements of two classical ORFs transcribed in tandem and encoding proteins consisting of two IRG units with a total protein molecular weight of around 94 kDa (Bekpen et al. 2005; Lilue et al. unpublished data). Most IRG genes in mice possess promoters with classical ISRE and GAS elements and are strongly inducible by IFN γ from low or undetectable resting levels. Exceptions from this paradigm are *Irgc*, which is expressed exclusively in the testis and is not IFN γ -inducible (Bekpen et al. 2005; Rohde et al. unpublished data), and *Irga6*, which in addition to a classical IFN γ -inducible promoter has a separate liver-specific promoter subtending a dedicated liver-specific 5'-untranslated exon (Zeng et al. 2009). In silico analyses suggest that there might also be a liver-specific alternative promoter in *Irgd*, though experimental evidence is lacking.

The IRG family evolves at a rate probably unprecedented in the GTPase superfamily. Even compared with two other families of IFN-inducible, large GTPases, i.e., the IFN γ -inducible GBPs and the type I IFN-inducible Mx proteins, the IRG genes and proteins show much higher sequence diversity both within and between species (Degrandi et al. 2007; Hunn unpublished data). Thus, the branch lengths separating individual members of the mouse IRG family are longer than the branch lengths between mouse and human members of the GBP or Mx family (Fig. 3). During the evolutionary divergence between mammalian species, IRG genes have rapidly lost their individual identities. Thus, the IRGB subfamily of GKS genes on mouse chromosome 11 can still be identified with a syntenic group of IRGB genes on rat chromosome 10, but most of the individual genes are no longer identifiable (Bekpen and Hunn unpublished data). The three mouse genes of the more slowly evolving GMS subfamily are individually identifiable in the rat but orthology with the three GMS proteins of the dog is not apparent (Bekpen et al. 2005). The only exception is the non-IFN-inducible, testis-expressed *Irgc* gene, which is highly conserved between human and mouse (Bekpen et al. 2005; Boehm et al. 1998).

Phylogenetic analyses revealed that multiple diverse IRG genes are present throughout the mammals and in reptiles, amphibians, fish, and cephalochordates, while no convincing homologs were detected in invertebrates (Bekpen et al. 2005; Hunn unpublished data; Li et al. 2009). Possibly related genes found in Cyanobacteria may suggest that IRG genes were acquired by a filter-feeding vertebrate ancestor by horizontal gene transfer (Bekpen et al. 2005).

Interestingly, the IRG resistance system also seems to have been repeatedly lost during evolution: To date, no IRG genes have been detected in any of the available bird genomes (chicken, zebra finch, duck, and turkey; Hunn unpublished data) and the number of IRG genes present in humans is strikingly reduced (Bekpen et al. 2005, 2009). The remaining two IRG genes, *IRGC* and *IRGM*, lack GAS and ISRE elements in their promoters, are not IFN-inducible, and may not possess immune function. Like its mouse homolog, human *IRGC* is expressed exclusively in the testis (Bekpen et al. 2005). Human *IRGM* is homologous to the three mouse GMS proteins. However, it is severely truncated relative to these and lacks the whole N- and C-terminal domains as well as part of the G domain. Nevertheless, the genetic association of *IRGM* with Crohn's disease in humans described above could reflect some immune function of human *IRGM*. All anthropoids (*Simiiformes*),

including chimp, gorilla, orangutan, macaque, and marmoset, possess only this reduced set of IRG genes (Bekpen et al. 2009; Hunn unpublished data). Apparently, the *IRGMORF* was disrupted by an Alu retroposition event in the anthropoid common ancestor and is a pseudogene in both the New World monkeys (*Platyrrhini*) and the Old World monkeys (*Cercopithecidae*) (Bekpen et al. 2009). A common ancestor of the apes (*Humanoidae*), however, acquired a functional promoter driving constitutive expression by insertion of an endogenous retrovirus. This insertion altered the transcription initiation, splicing, and expression profile resulting in “resurrection” of a protein-coding *IRGM* gene, albeit in a truncated form (Bekpen et al. 2005, 2009).

In contrast to the anthropoids, the lower primates, like most mammals, contain a diverse set of immune-type IRG GTPases (Bekpen et al. 2009; Hunn unpublished data). Thus, the resistance IRGs must have been lost between the divide of the *Haplorrhini* (tarsier and anthropoids) and the *Strepsirrhini* (loris and lemurs) perhaps 60 million years ago and the division of the *Hominoidea* and the Old World monkeys (*Cercopithecidae*) from the New World monkeys (*Platyrrhini*) 30–40 million years ago (Bekpen et al. 2009; Hunn unpublished data).

Most other mammalian species analyzed possess a diverse set of “immune-type” IRG genes, but so far GMS proteins have been detected only in eutherian mammals (Hunn unpublished data). This opens up the questions of whether the complex regulatory interactions that drive the IRG resistance system in mice are a mammalian invention and whether the IRG systems in the other vertebrates and in the cephalochordates are regulated differently. This question of how negative regulation is achieved is a key issue for all large, self-activating GTPases like dynamin, Mx proteins, and GBPs and is an issue that remains largely unresolved for these proteins.

Host-pathogen coevolution

Research on *Chlamydia* and our own recent analyses on *T. gondii* resistance indicate that there is a correlation between pathogen virulence and the presence of a certain set of IRG proteins (Bernstein-Hanley et al. 2006; Coers et al. 2008; Lilue et al unpublished data; Steinfeldt et al unpublished data). The important work of Coers et al. 2008 on the IFN γ -dependent control of *C. trachomatis* revealed that the genetic susceptibility of C3H/HeJ inbred mice depends on a sequence polymorphism upstream of the *Irgb10* gene, leading to a barely detectable expression of the protein (Bernstein-Hanley et al. 2006). Furthermore, the IRG system efficiently controls the human pathogenic strain *C. trachomatis*, while the mouse pathogenic strain *C. muridarum* efficiently counteracts the IRG system and replicates freely (Al-Zeer et al. 2009; Coers et al. 2008). Similarly, the IRG system efficiently controls avirulent but not virulent *T. gondii* strains (see above) (Khaminets et al. 2010; Zhao et al. 2009a, c). Further analysis has provided evidence for polymorphisms in IRG genes in laboratory strains of *Mus musculus* and in wild and wild-derived inbred mice from diverse sources (Lilue et al. unpublished data). This polymorphism in components of the IRG resistance system may result in differential resistance to individual parasite strains. Sterile immunity of the inbred Lewis rat strain against *T. gondii* has been reported (Cavaillès et al. 2006). The causative polymorphic locus has not yet been identified but is linked to the IRG system on chromosome 10. Taken together, the data suggest that diversity in the IRG genes might be driven by allele-specific host-pathogen interactions. Recently, the virulent allelic product of the polymorphic rho-kinase ROP18 (Khan et al. 2009), known to contribute to the virulence of type I *T. gondii* strains for mice (Taylor et al. 2006), has been shown to phosphorylate and inactivate certain IRG proteins at the *T. gondii* PV in C57BL/6 strain mice (Fentress et al. unpublished data; Steinfeldt et al. unpublished data). Such action by

polymorphic parasite virulence factors could contribute to polymorphism in the host repertoire of IRG resistance.

Conclusion

The IRG system is a powerful resistance mechanism in mammals against certain intracellular pathogens. Nevertheless, the function of the IRG system can apparently be dispensed with, as in the last stages of the human evolutionary lineage, and apparently also in birds. There is much work to be done to define the mode of action of the IRG proteins in any pathogen-resistance system. An obvious issue is whether the mechanism of action is the same for such different pathogens as *Chlamydia* and *Toxoplasma*. The IRG system appears to be especially highly developed in the mouse and probably also in other small rodents. There is striking evidence for polymorphism of ROP18 (Khan et al. 2009) and other putative virulence factors among wild *T. gondii* strains and a complex population structure worldwide (Boyle et al. 2006; Khan et al. 2007; Sibley and Ajioika 2008), suggesting that the fitness of specific *T. gondii* genotypes has been changing in recent times, perhaps through the increasing importance of the domestic cat as a definitive host and typical cat prey such as *Mus musculus* as the intermediate hosts of choice. Since IRG proteins are almost certainly under ongoing selection from *T. gondii* in wild mice, the relative clarity of the system lends itself to a detailed analysis of recent evolution in a well-defined host-pathogen system in mammals.

Acknowledgments

JCH and JPH were supported by SFB635, SFB670, SFB680 and SPP1399 from the Deutsche Forschungsgemeinschaft; and CGF and AS were supported by the Intramural Research Program of the NIAID, NIH. We are grateful to our colleague Gregory Taylor for his pioneering work on the IRG system.

References

- Al-Zeer MA, Al-Younes HM, Braun PR, Zerrahn J, Meyer TF. IFN-gamma-inducible Irga6 mediates host resistance against *Chlamydia trachomatis* via autophagy. PLoS One. 2009; 4:e4588. [PubMed: 19242543]
- Araujo FG, Williams DM, Grumet FC, Remington JS. Strain-dependent differences in murine susceptibility to toxoplasma. Infect Immun. 1976; 13(5):1528–1530. [PubMed: 1270155]
- Bashkirov PV, Akimov SA, Evseev AI, Schmid SL, Zimmerberg J, et al. GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. Cell. 2008; 135:1276–1286. [PubMed: 19084269]
- Bekpen C, Hunn JP, Rohde C, Parvanova I, Guethlein L, et al. The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage. Genome Biol. 2005; 6:R92. [PubMed: 16277747]
- Bekpen C, Marques-Bonet T, Alkan C, Antonacci F, Leogrande MB, et al. Death and resurrection of the human IRGM gene. PLoS Genet. 2009; 5 e1000403.
- Bernstein-Hanley I, Coers J, Balsara ZR, Taylor GA, Starnbach MN, et al. The p47 GTPases Irgp and Irgb10 map to the *Chlamydia trachomatis* susceptibility locus *Ctrq-3* and mediate cellular resistance in mice. Proc Natl Acad Sci USA. 2006; 103:14092–14097. [PubMed: 16959883]
- Binns DD, Barylko B, Grichine N, Atkinson MA, Helms MK, et al. Correlation between self-association modes and GTPase activation of dynamin. J Protein Chem. 1999; 18:277–290. [PubMed: 10395446]
- Boehm U, Guethlein L, Klamp T, Ozbek K, Schaub A, et al. Two families of GTPases dominate the complex cellular response to IFN-gamma. J Immunol. 1998; 161:6715–6723. [PubMed: 9862701]
- Boguski MS, McCormick F. Proteins regulating Ras and its relatives. Nature. 1993; 366:643–654. [PubMed: 8259209]

- Boothroyd JC, Grigg ME. Population biology of *Toxoplasma gondii* and its relevance to human infection: do different strains cause different disease? *Curr Opin Microbiol.* 2002; 5:438–442. [PubMed: 12160866]
- Bougneres L, Helft J, Tiwari S, Vargas P, Chang BH, et al. A role for lipid bodies in the cross-presentation of phagocytosed antigens by MHC class I in dendritic cells. *Immunity.* 2009; 31:232–244. [PubMed: 19699172]
- Boyle JP, Rajasekar B, Saeij JP, Ajioka JW, Berriman M, et al. Just one cross appears capable of dramatically altering the population biology of a eukaryotic pathogen like *Toxoplasma gondii*. *Proc Natl Acad Sci USA.* 2006; 103:10514–10519. [PubMed: 16801557]
- Butcher BA, Greene RI, Henry SC, Annecharico KL, Weinberg JB, Denkers EY, Sher A, Taylor GA. p47 GTPases regulate *Toxoplasma gondii* survival in activated macrophages. *Infect Immun.* 2005; 73:3278–3286. [PubMed: 15908352]
- Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, et al. A key role for autophagy and the autophagy gene *Atg16l1* in mouse and human intestinal Paneth cells. *Nature.* 2008; 456:259–263. [PubMed: 18849966]
- Carlow DA, Marth J, Clark-Lewis I, Teh HS. Isolation of a gene encoding a developmentally regulated T cell-specific protein with a guanine nucleotide triphosphate-binding motif. *J Immunol.* 1995; 154:1724–1734. [PubMed: 7836757]
- Cavaillès P, Bisanz C, Papapietro O, Colacios C, Sergent V, et al. The rat *Toxo1* locus controls the outcome of the toxoplasmic infection according to a mendelian mode. *Med Sci (Paris).* 2006; 22:679–680. [PubMed: 16962031]
- Che N, Li S, Gao T, Zhang Z, Han Y, et al. Identification of a novel IRGM promoter single nucleotide polymorphism associated with tuberculosis. *Clin Chim Acta.* 2010; 411(21–22):1645–1649. [PubMed: 20547146]
- Chen X, Du X, Zhang M, Zhang D, Ji M, et al. IFN-inducible p47 GTPases display differential responses to *Schistosoma japonicum* acute infection. *Cell Mol Immunol.* 2010; 7(1):69–76. [PubMed: 20029464]
- Coers J, Bernstein-Hanley I, Grotzky D, Parvanova I, Howard JC, et al. *Chlamydia muridarum* evades growth restriction by the IFN-gamma-inducible host resistance factor Irgb10. *J Immunol.* 2008; 180:6237–6245. [PubMed: 18424746]
- Collazo CM, Yap GS, Sempowski GD, Lusby KC, Tessarollo L, et al. Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. *J Exp Med.* 2001; 194:181–188. [PubMed: 11457893]
- Consortium WTCC. Genome-wide association study of 14, 000 cases of seven common diseases and 3, 000 shared controls. *Nature.* 2007; 447:661–678. [PubMed: 17554300]
- Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, et al. Genome-wide association study of CNVs in 16, 000 cases of eight common diseases and 3, 000 shared controls. *Nature.* 2010; 464:713–720. [PubMed: 20360734]
- Degrandi D, Konermann C, Beuter-Gunia C, Kresse A, Wurthner J, et al. Extensive characterization of IFN-induced GTPases mGBP1 to mGBP10 involved in host defense. *J Immunol.* 2007; 179:7729–7740. [PubMed: 18025219]
- de Souza AP, Tang B, Tanowitz HB, Factor SM, Shtutin V, Shirani J, Taylor GA, Weiss LM, Jelicks LA. Absence of interferon-gamma-inducible gene IGTP does not significantly alter the development of chagasic cardiomyopathy in mice infected with *Trypanosoma cruzi* (Brazil strain). *J Parasitol.* 2003; 89:1237–1239. [PubMed: 14740917]
- Feng CG, Collazo-Custodio CM, Eckhaus M, Hieny S, Belkaid Y, et al. Mice deficient in LRG-47 display increased susceptibility to mycobacterial infection associated with the induction of lymphopenia. *J Immunol.* 2004; 172:1163–1168. [PubMed: 14707092]
- Feng CG, Weksberg DC, Taylor GA, Sher A, Goodell MA. The p47 GTPase Lrg-47 (Irgm1) links host defense and hematopoietic stem cell proliferation. *Cell Stem Cell.* 2008a; 2:83–89. [PubMed: 18371424]
- Feng CG, Zheng L, Jankovic D, Bafica A, Cannons JL, et al. The immunity-related GTPase Irgm1 promotes the expansion of activated CD4+ T cell populations by preventing interferon-gamma-induced cell death. *Nat Immunol.* 2008b; 9:1279–1287. [PubMed: 18806793]

- Gao S, von der Malsburg A, Paeschke S, Behlke J, Haller O, et al. Structural basis of oligomerization in the stalk region of dynamin-like MxA. *Nature*. 2010; 465:502–506. [PubMed: 20428112]
- Ghosh A, Uthaiiah R, Howard J, Herrmann C, Wolf E. Crystal structure of IIGP1: a paradigm for interferon-inducible p47 resistance GTPases. *Mol Cell*. 2004; 15:727–739. [PubMed: 15350217]
- Gilly M, Wall R. The IRG-47 gene is IFN-gamma induced in B cells and encodes a protein with GTP-binding motifs. *J Immunol*. 1992; 148:3275–3281. [PubMed: 1578148]
- Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, et al. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell*. 2004; 119:753–766. [PubMed: 15607973]
- Halonen SK. Role of autophagy in the host defense against *Toxoplasma gondii* in astrocytes. *Autophagy*. 2009; 5:268–269. [PubMed: 19139630]
- Halonen SK, Taylor GA, Weiss LM. Gamma interferon-induced inhibition of *Toxoplasma gondii* in astrocytes is mediated by IGTP. *Infect Immun*. 2001; 69:5573–5576. [PubMed: 11500431]
- Henry SC, Daniell X, Indaram M, Whitesides JF, Sempowski GD, et al. Impaired macrophage function underscores susceptibility to Salmonella in mice lacking Irgm1 (LRG-47). *J Immunol*. 2007; 179:6963–6972. [PubMed: 17982087]
- Henry SC, Daniell XG, Burroughs AR, Indaram M, Howell DN, et al. Balance of Irgm protein activities determines IFN- γ -induced host defense. *J Leukoc Biol*. 2009; 85:877–885. [PubMed: 19176402]
- Henry SC, Traver M, Daniell X, Indaram M, Oliver T, et al. Regulation of macrophage motility by Irgm1. *J Leukoc Biol*. 2010; 87(2):333–343. [PubMed: 19920210]
- Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis*. 1995; 172:1561–1566. [PubMed: 7594717]
- Hunn JP, Howard JC. The mouse resistance protein Irgm1 (LRG-47): A regulator or an effector of pathogen defense? *PLoS Pathog*. 2010; 6:e1001008.
- Hunn JP, Koenen-Waisman S, Papic N, Schroeder N, Pawlowski N, et al. Regulatory interactions between IRG resistance GTPases in the cellular response to *Toxoplasma gondii*. *EMBO J*. 2008; 27:2495–2509. [PubMed: 18772884]
- Intemann CD, Thye T, Niemann S, Browne EN, Amanua Chinbuah M, et al. Autophagy gene variant IRGM-261T contributes to protection from tuberculosis caused by *Mycobacterium tuberculosis* but not by *M. africanum* strains. *PLoS Pathog*. 2009; 5:e1000577.
- Jones DT, Taylor WR, Thornton JM. The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci*. 1992; 8:275–282. [PubMed: 1633570]
- Khaminets A, Hunn JP, Koenen-Waisman S, Zhao YO, Preukschat D, et al. Coordinated loading of IRG resistance GTPases onto the *Toxoplasma gondii* parasitophorous vacuole. *Cell Microbiol*. 2010; 12(7):939–961. [PubMed: 20109161]
- Khan A, Fux B, Su C, Dubey JP, Darde ML, et al. Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. *Proc Natl Acad Sci USA*. 2007; 104:14872–14877. [PubMed: 17804804]
- Khan A, Taylor S, Ajioka JW, Rosenthal BM, Sibley LD. Selection at a single locus leads to widespread expansion of *Toxoplasma gondii* lineages that are virulent in mice. *PLoS Genet*. 2009; 5:e1000404.
- Koga R, Hamano S, Kuwata H, Atarashi K, Ogawa M, Hisaeda H, Yamamoto M, Akira S, Himeno K, Matsumoto M, Takeda K. TLR-dependent induction of IFN-beta mediates host defense against *Trypanosoma cruzi*. *J Immunol*. 2006; 177:7059–7066. [PubMed: 17082622]
- Konen-Waisman S, Howard JC. Cell-autonomous immunity to *Toxoplasma gondii* in mouse and man. *Microbes Infect*. 2007; 9:1652–1661. [PubMed: 18024121]
- Lafuse WP, Brown D, Castle L, Zwilling BS. Cloning and characterization of a novel cDNA that is IFN-gamma-induced in mouse peritoneal macrophages and encodes a putative GTP-binding protein. *J Leukoc Biol*. 1995; 57:477–483. [PubMed: 7884320]
- Li G, Zhang J, Sun Y, Wang H, Wang Y. The evolutionarily dynamic IFN-inducible GTPase proteins play conserved immune functions in vertebrates and cephalochordates. *Mol Biol Evol*. 2009; 26:1619–1630. [PubMed: 19369598]

- Ling YM, Shaw MH, Ayala C, Coppens I, Taylor GA, et al. Vacuolar and plasma membrane stripping and autophagic elimination of *Toxoplasma gondii* in primed effector macrophages. *J Exp Med*. 2006; 203:2063–2071. [PubMed: 16940170]
- MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science*. 2003; 302:654–659. [PubMed: 14576437]
- Martens S, Sabel K, Lange R, Uthaiiah R, Wolf E, et al. Mechanisms regulating the positioning of mouse p47 resistance GTPases LRG-47 and IIGP1 on cellular membranes: retargeting to plasma membrane induced by phagocytosis. *J Immunol*. 2004; 173:2594–2606. [PubMed: 15294976]
- Martens S, Parvanova I, Zerrahn J, Griffiths G, Schell G, et al. Disruption of *Toxoplasma gondii* parasitophorous vacuoles by the mouse p47-resistance GTPases. *PLoS Pathog*. 2005; 1:e24. [PubMed: 16304607]
- McCarroll SA, Huett A, Kuballa P, Chilewski SD, Landry A, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet*. 2008; 40:1107–1112. [PubMed: 19165925]
- Melzer T, Duffy A, Weiss LM, Halonen SK. The gamma interferon (IFN-gamma)-inducible GTP-binding protein IGTP is necessary for toxoplasma vacuolar disruption and induces parasite egression in IFN-gamma-stimulated astrocytes. *Infect Immun*. 2008; 76:4883–4894. [PubMed: 18765738]
- Miyairi I, Tatireddigari VR, Mahdi OS, Rose LA, Belland RJ, et al. The p47 GTPases Iigp2 and Irgb10 regulate innate immunity and inflammation to murine *Chlamydia psittaci* infection. *J Immunol*. 2007; 179:1814–1824. [PubMed: 17641048]
- Nelson DE, Virok DP, Wood H, Roshick C, Johnson RM, Whitmire WM, Crane DD, Steele-Mortimer O, Kari L, McClarty G, Caldwell HD. Chlamydial IFN-gamma immune evasion is linked to host infection tropism. *Proc Natl Acad Sci USA*. 2005; 102:10658–10663. [PubMed: 16020528]
- Orlowsky A. Toxoplasma-induced autophagy: A window into nutritional futile cycles in mammalian cells? *Autophagy*. 2009; 5(3):404–406. [PubMed: 19305153]
- Papic N, Hunn JP, Pawlowski N, Zerrahn J, Howard JC. Inactive and active states of the interferon-inducible resistance GTPase, Irga6, in vivo. *J Biol Chem*. 2008; 283:32143–32151. [PubMed: 18784077]
- Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, et al. Sequence variants in the autophagy gene *IRGM* and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet*. 2007; 39:830–832. [PubMed: 17554261]
- Pitossi F, Blank A, Schroder A, Schwarz A, Hussi P, et al. A functional GTP-binding motif is necessary for antiviral activity of Mx proteins. *J Virol*. 1993; 67:6726–6732. [PubMed: 8411374]
- Praefcke GJ, McMahon HT. The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol*. 2004; 5:133–147. [PubMed: 15040446]
- Praefcke GJ, Kloepe S, Benschied U, Lilie H, Prakash B, et al. Identification of residues in the human guanylate-binding protein 1 critical for nucleotide binding and cooperative GTP hydrolysis. *J Mol Biol*. 2004; 344:257–269. [PubMed: 15504415]
- Prakash B, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C. Structure of human guanylate-binding protein 1 representing a unique class of GTP-binding proteins. *Nature*. 2000; 403:567–571. [PubMed: 10676968]
- Pucadyil TJ, Schmid SL. Real-time visualization of dynamin-catalyzed membrane fission and vesicle release. *Cell*. 2008; 135:1263–1275. [PubMed: 19084268]
- Santiago HC, Feng CG, Bafica A, Roffe E, Arantes RM, et al. Mice deficient in LRG-47 display enhanced susceptibility to *Trypanosoma cruzi* infection associated with defective hemopoiesis and intracellular control of parasite growth. *J Immunol*. 2005; 175:8165–8172. [PubMed: 16339555]
- Sibley LD, Ajioka JW. Population structure of *Toxoplasma gondii*: clonal expansion driven by infrequent recombination and selective sweeps. *Annu Rev Microbiol*. 2008; 62:329–351. [PubMed: 18544039]
- Sigal IS, Gibbs JB, D'Alonzo JS, Temeles GL, Wolanski BS, et al. Mutant ras-encoded proteins with altered nucleotide binding exert dominant biological effects. *Proc Natl Acad Sci USA*. 1986; 83:952–956. [PubMed: 3513168]

- Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science*. 2006; 313:1438–1441. [PubMed: 16888103]
- Sorace JM, Johnson RJ, Howard DL, Drysdale BE. Identification of an endotoxin and IFN-inducible cDNA: possible identification of a novel protein family. *J Leukoc Biol*. 1995; 58:477–484. [PubMed: 7561525]
- Sweitzer SM, Hinshaw JE. Dynamin undergoes a GTP-dependent conformational change causing vesiculation. *Cell*. 1998; 93:1021–1029. [PubMed: 9635431]
- Taylor GA. IRG proteins: key mediators of interferon-regulated host resistance to intracellular pathogens. *Cell Microbiol*. 2007; 9:1099–1107. [PubMed: 17359233]
- Taylor GA, Jeffers M, Largaespada DA, Jenkins NA, Copeland NG, et al. Identification of a novel GTPase, the inducibly expressed GTPase, that accumulates in response to interferon gamma. *J Biol Chem*. 1996; 271:20399–20405. [PubMed: 8702776]
- Taylor GA, Stauber R, Rulong S, Hudson E, Pei V, et al. The inducibly expressed GTPase localizes to the endoplasmic reticulum, independently of GTP binding. *J Biol Chem*. 1997; 272:10639–10645. [PubMed: 9099712]
- Taylor GA, Collazo CM, Yap GS, Nguyen K, Gregorio TA, et al. Pathogen-specific loss of host resistance in mice lacking the IFN-gamma-inducible gene *IGTP*. *Proc Natl Acad Sci USA*. 2000; 97:751–755. [PubMed: 10639151]
- Taylor GA, Feng CG, Sher A. p47 GTPases: regulators of immunity to intracellular pathogens. *Nat Rev Immunol*. 2004; 4:100–109. [PubMed: 15040583]
- Taylor S, Barragan A, Su C, Fux B, Fentress SJ, et al. A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen *Toxoplasma gondii*. *Science*. 2006; 314:1776–1780. [PubMed: 17170305]
- Taylor GA, Feng CG, Sher A. Control of IFN-gamma-mediated host resistance to intracellular pathogens by immunity-related GTPases (p47 GTPases). *Microbes Infect*. 2007; 9:1644–1651. [PubMed: 18023232]
- Tuma PL, Collins CA. Activation of dynamin GTPase is a result of positive cooperativity. *J Biol Chem*. 1994; 269:30842–30847. [PubMed: 7983015]
- Uthai RC, Praefcke GJ, Howard JC, Herrmann C. IIGP1, an interferon-gamma-inducible 47-kDa GTPase of the mouse, showing cooperative enzymatic activity and GTP-dependent multimerization. *J Biol Chem*. 2003; 278:29336–29343. [PubMed: 12732635]
- Wang Y, Weiss LM, Orlofsky A. Host cell autophagy is induced by *Toxoplasma gondii* and contributes to parasite growth. *J Biol Chem*. 2009; 284:1694–1701. [PubMed: 19028680]
- Warnock DE, Hinshaw JE, Schmid SL. Dynamin self-assembly stimulates its GTPase activity. *J Biol Chem*. 1996; 271:22310–22314. [PubMed: 8798389]
- Zeng J, Parvanova IA, Howard JC. A dedicated promoter drives constitutive expression of the cell-autonomous immune resistance GTPase, Irga6 (IIGP1), in mouse liver. *PLoS One*. 2009; 4:e6787. [PubMed: 20368812]
- Zhao Z, Fux B, Goodwin M, Dunay IR, Strong D, et al. Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. *Cell Host Microbe*. 2008; 4:458–469. [PubMed: 18996346]
- Zhao Y, Ferguson DJ, Wilson DC, Howard JC, Sibley LD, et al. Virulent *Toxoplasma gondii* evade immunity-related GTPase-mediated parasite vacuole disruption within primed macrophages. *J Immunol*. 2009a; 182:3775–3781. [PubMed: 19265156]
- Zhao YO, Khaminets A, Hunn JP, Howard JC. Disruption of the *Toxoplasma gondii* parasitophorous vacuole by IFN-gamma-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. *PLoS Pathog*. 2009b; 5:e1000288.
- Zhao YO, Rohde C, Lilue JT, Konen-Waisman S, Khaminets A, et al. *Toxoplasma gondii* and the Immunity-Related GTPase (IRG) resistance system in mice: a review. *Mem Inst Oswaldo Cruz*. 2009c; 104:234–240. [PubMed: 19430648]

Fig. 1. Crystal structure of Irga6. GDP-bound Irga6 monomer (ribbon presentation) is shown with the G domain (S1–H5) colored in *light blue* and the N- and C-terminal helical regions colored in *cyan* (αA–αC) and *dark blue* (αF–αL). The linker helix αE connecting the G domain and C-terminal helical region is shown in *gray*. GDP and Mg²⁺ are shown as atomic stick figure and *yellow* sphere, respectively (reprinted from Ghosh et al. 2004 with permission from Elsevier)

Fig. 2.

Irga6 PV localization, rupture, and necrosis. Host cells die after disruption of the PVM and death of the *T. gondii*. Mouse embryonic fibroblasts were treated with 200 U/ml IFN γ and cotransfected with Irga6-ctag1-EGFP and pDsRed constructs for 24 h. Cells were then infected with ME49 strain *T. gondii* at multiplicities of infection between 5 and 10 for 1 h and observed microscopically by time-lapse photography with 5-min intervals. Three PVs were observed within this cell when the experiment started and selected frames are shown. Two Irga6-ctag1-EGFP-positive PVMs were disrupted (*arrows*) and the respective parasites became permeable to mDsRed (*arrowheads*). At 2:30 after infection, the mDsRed signal suddenly disappeared from the host cell accompanied by a drastic change in host cell morphology shown in phase-contrast images. *Scale bar* = 10 μ m (1:10: time in h:min) (adapted from Zhao et al. 2009b)

Fig. 3.

Phylogenetic tree of human and mouse IFN-inducible GTP-ases. Neighbor-joining consensus bootstrapped tree (100 replicates) based on a ClustalW protein alignment of full-length IFN-inducible GTPases manually fixed on the nucleotide-binding motifs (*left*: unrooted; *right*: rooted). Known pseudogenes were excluded from the analysis. The phylogenetic analysis was performed applying the JTT + I + G amino acid replacement matrix (Jones et al. 1992). This tree allows the comparison of the diversity occurring within the individual GTPase families. Note that the apparent distance of the three GTPase families from each other is slightly distorted due to the different lengths of the sequences. Also note that MM Gbp8 lacks exon 6 (82 amino acids). MM: *Mus musculus* (C57BL/6); HS: *Homo sapiens*. Bootstrap values are given on the branches of the unrooted tree

Table 1

IRG protein-mediated resistance against pathogens

Pathogen	In vivo (ko mice/strain differences)								Cell autonomous effect	References
	wt	IFN γ ^{-/-}	Irgm1 ^{-/-}	Irgm3 ^{-/-}	Irga6 ^{-/-}	Irgd ^{-/-}	Irgm1/3 ^{-/-}	Strain diff		
<i>Toxoplasma gondii</i>	R	S	S	S	S	S	S	ND	m1, m3, a6, b6	Ling et al. (2006), Martens et al. (2005), Butcher et al. (2005), Collazo et al. (2001), Taylor et al. (2000)
<i>Leishmania major</i>	R	S	S	S	R	ND	ND	ND	ND	Taylor et al. (2004), Liesenfeld et al. (unpublished)
<i>Trypanosoma cruzi</i>	R	S	S	R	ND	ND	ND	ND	m1, d	de Souza et al. (2003), Santiago et al. (2005), Koga et al. (2006)
<i>Chlamydia trachomatis</i>	R	S	S	S	R	ND	ND	b10	m1, m3, b10, a6 (conflicting)	Nelson et al. (2005), Bernstein-Hanley et al. (2006), Coers et al. (2008), Al-Zeer et al. (2009)
<i>C. psittaci</i>	R	S	ND	S	ND	ND	ND	m2, b10	m2	Miyairi et al. (2007)
<i>Listeria monocytogenes</i>	R	S	S	R	ND	ND	ND	ND	ND	Collazo et al. (2001), Liesenfeld et al. (unpublished)
<i>Legionella pneumophila</i>	R	S	ND	ND	ND	ND	ND	ND	m3 (m1 & m1/3 ko normal)	Henry et al. (2009)
<i>Mycobacterium avium</i>	R	S	S	R	ND	ND	R	ND	ND	Feng et al. (2004, 2008b, unpublished data)
<i>M. tuberculosis</i>	R	S	S	R	ND	R	R	ND	m1	MacMicking et al. (2003), Henry et al. (2009)
<i>Salmonella typhimurium</i>	R	S	S	R	ND	R	R	ND	m1 (m3 & m1/3 ko normal)	Henry et al. (2007, 2009)
<i>Schistosoma mansoni</i>	R	R	R	ND	ND	ND	ND	ND	ND	Feng (2008b)
<i>S. japonicum</i>	R	No effect	ND	No effect	ND	Fewer eggs	ND	ND	ND	Chen et al. (2010)
MCMV	R	S	R	R	ND	R	ND	ND	ND	Taylor et al. (2000), Collazo et al. (2001)
Ebola virus	R	Weak S	ND	R	ND	ND	ND	ND	ND	Taylor et al. (2000)

R resistant; S susceptible; ND not determined; wt wild type C57Bl/6 mice; strain diff resistance differences between mouse strains due to IRGs; m1 = Irgm1; m2 = Irgm2; m3 = Irgm3; a6 = Irga6; b6 = Irgb6; d = Irgd; m1/3 = Irgm1/Irgm3

Cell-autonomous effects were shown in either knockout (ko) cells, in knockdown, or overexpression studies